 doi:10.20944/preprints202207.0198.v1

Article

Association of serum and urinary magnesium levels with body composition and inflammatory markers in patients with non-dialysis chronic kidney disease: a longitudinal approach

Raimunda S. C. Dias[1*], Dyego J. A. Brito[2], Cleodice A. Martins[3], Elton J. F. Santos[4], Erika C. R. L. Carneiro[5], Elisângela M. dos Santos[6], Denizar V. Araújo[7], Natalino Salgado Filho[8], Mário Bernardo Filho[9]

[1*] Center for Prevention of Kidney Diseases, University Hospital of the Federal University of Maranhão, São Luís, MA, Brazil; sheylak75@yahoo.com.br

[2] Center for Prevention of Kidney Diseases, University Hospital of the Federal University of Maranhão, São Luís, MA, Brazil; djabrito@uol.com.br

[3] 1. Postgraduate Program Nutrition and Health, Health Sciences, Federal University of Espírito Santo; cleoalves.01@hotmail.com

[4] Center for Prevention of Kidney Diseases, University Hospital of the Federal University of Maranhão, São Luís, MA, Brazil; eltonfreitas86@yahoo.com.br

[5] Center for Prevention of Kidney Diseases, University Hospital of the Federal University of Maranhão, São Luís, MA, Brazil; eltonfreitas86@yahoo.com.br

[6] Nursing Department, Federal University of Maranhão, São Luís, MA, Brazil. elismilhomem@hotmail.com

[7] Department of Internal Medicine, University of the State of Rio de Janeiro, RJ, Brazil. denizarvianna@gmail.com

[8] Department of Medicine I, Federal University of Maranhão, São Luís, MA, Brazil. natalinosalgadofilho@uol.com.br

[9] Laboratory of Mechanical Vibrations and Integrative Practices, Department of Biophysics and Biometrics, Institute of Biology Roberto Alcântara Gomes and Polyclinic Américo Piquet Carneiro, University of the State of Rio de Janeiro, RJ, Brazil. bernardofilhom@gmail.com

*Correspondence: Email: sheylak75@yahoo.com.br; Phone: +55 98 981545296

Abstract: (1) Background: Reduced magnesium (Mg) levels may be associated with a faster decline in renal function. The aim of this study was to evaluate the association of serum and urinary Mg levels with body composition and inflammatory markers; (2) Methods: Longitudinal study with patients with chronic kidney disease undergoing non-dialysis treatment in stages 3A, 3B and 4. Venous samples were collected after a 12-hour night fast. Body composition was evaluated by Double X-Ray Emission Absorptiometry and Air Displacement Plethysmography; (3) Results: The sample consisted of 134 patients. In the adjusted linear regression model, uric acid, percentage of lean mass and ali-mentar intake of Mg were positively associated with the sergic Mg. Triglyceride levels, WC and fat mass percentage were negatively associated with serum Mg. For the Mg urinal, in the adjusted model, the eGFR (estimated glomerular filtration rate), IL (interleukin 6), food intake of Mg and the percentage of lean mass showed a positive correlation.; (4) Conclusions: Serum Mg levels were positively associated with lean mass and negatively with total and central body fat and urinary Mg was positively associated with IL6 and lean mass.

Keywords: Magnesium deficiency; Body composition; Chronic kidney failure.

 doi:10.20944/preprints202207.0198.v1

1. Introduction

Chronic kidney disease (CKD) is a relevant public health problem and is characterized by the slow, progressive and irreversible loss of kidney function, having great impacto in the lives of patients, in health services, and in society [1,2].

In CKD patients, inflammation is highly prevalent and is activated by multiple mechanisms [3-5]. There is evidence of activation of the immune system in early and late stages of CKD [6], and minerals have been the subject of several studies to try elucidating mechanisms involved in this pathogenesis [7,8].

Magnesium (Mg) has attracted the interest of researchers, as it is a crucial cofactor for hundreds of enzymatic reactions and biological processes. It is necessary for processes of oxidative phosphorylation, energy production reactions, protein synthesis, glycolysis and nucleic acid synthesis and stability [9,10].

Reduced Mg levels may be associated with a faster decline in renal function, mortality, or adverse cardiovascular outcome in CKD. The mechanisms that explain this relationship still need to be clarified, but endothelial dysfunction, inflammation, insulin resistance, oxidative stress and hypertension may be involved [7,8].

In addition, studies have shown that there is a relationship between Mglevels and obesity [11-13]. In this sense, the National Health and Nutrition Examination Survey (NHANES) highlights that Mg deficit is more prevalent in individuals with body mass index (BMI) in the obesity range [14]. The CARDIA longitudinal research concluded that Mg intake is inversely associated with obesity and C-reactive protein (CRP) levels [15].

Scientific evidence has shown that the reduction of Mg levels favors the manifestation of low-grade chronic inflammation and may be associated with a faster decline in renal function and higher risk of obesity [7, 9, 11, 14]. Although explanations have been proposed with the objective of clarifying the role of mineral in these disorders, the mechanisms are not yet elucidated. Therefore, further studies on this subject might provide biochemical and pathophysiological basis to try to explain the role of Mg in the prevention and treatment of obesity, inflammation mitigation and progression of CKD.

Thus, the aim of this study was to investigate the association of serum and urinary mg levels with body composition and inflammatory markers in patients with non-dialysis CKD. The hypothesis is that reduced levels of serum and urinary Mg are associated with higher percentage of body fat, lower lean mass and higher concentration of inflammatory markers.

2. Materials and Methods

This is a longitudinal study, prospective for a period of 24 months, with a sample of patients with CKD undergoing non-dialysis treatment, under follow-up at the *Centro de Prevenção de Doenças Renais do Hospital Universitário da Universidade Federal do Maranhão (CPDR-HUUFMA).* The study approved by the Research Ethics Committee of the Universitário HUUFMA (opinion: 2.727.940) and meets the requirements required by The National Health Council Resolution n. 466/12 and its complements for research involving human beings.

Included in the study: patients with CKD in stages 3A, 3B and 4, of both sexes, aged 20 years or older and who maintained regular follow-up in 2017 at CPDR-HUUFMA. Not included: pregnant women; people with members' support; autoimmune diseases, consumptive diseases and urinary infection.

2.1. Data collection

The study was developed in three steps: t1 (inclusion), t2 (12 months) and t3 (24 months). At the time, the study participants answered a standardized questionnaire, containing questions related to demographic, socioeconomic characteristics, lifestyle and history of diseases, in addition to the drug therapy in use.

Age was categorized into 20-44 years, 45-59 years and ≥60 years and family income into minimum wage (MW): < 1MW; 1-2 MW and >2 MW. Marital status was classified as

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 July 2022 doi:10.20944/preprints202207.0198.v1

a marital home and single/separated/widowed. Skin color was self-reported [16] and classifi-each in white; black or brown and others. Schooling was evaluated in years of school attendance and classified as: ≤ 8 and > 8 years. Every patient who declared to be a smoker or consumed alcoholic at the time of the interview was considered a smoker and/or alcoholic at the time of the interview, regardless of the amount consumed.

To assess the level of physical activity of the participants, the number of days, minutes and type of physical activities performed was discussed. Patients who performed at least 150 minutes of semanal physical activity were considered physically active [17].

Patients with hypertension and/or diabetes mellitus with previous medical diagnosis and/or who were using specific medication were considered. Blood pressure measurement was performed using the oscillometric method using the Omron 705-IT (Japan) device and followed the recommendations of the Brazilian Guidelines on Arterial Hypertension – 2020 [18]. Three measurements were performed, with an interval of 1 to 2 minutes and additional measurements when the first two readings differed > 10 mmHg [18].

Venous samples were collected after a 12-hour night fast and included: creatinine, magnesium, calcium, sodium, uric acid, phosphorus, parathyroid hormone (PTH), vitamin D, lipid profile (triglycerides, total cholesterol, HDL cholesterol and LDL cholesterol), fasting glycemia, alkaline phosphatase, complete blood count, interleukin-6 (IL-6), TNF and ultrasensitive C-reactive protein (PCRus).

Creatinine levels (serum and urinary) were determined by the colorimetric method (Jaffé reaction). The lipid profile (total cholesterol and HDL cholesterol) was dosed by the colorimetric enzymatic method and the LDL cholesterol estimated by friedewald formula [19]. Magnesium levels (serum and urinary) were evaluated by the colorimetric method with chlorophosphane III. Phosphorus was dosed using the UV-automated molybderate method and vitamin D by electrochemiluminescence. Fasting glycemia was evaluated by UV hexokinase and hemoglobin by flow cytometry. The levels of PCRus were determinated by turbidimetry. Alkaline phosphatase was determined by enzymatic colorimetric (IFCC) method. Hematological parameters were analyzed using the Advia 120 System (Siemens AG, Germany) and the other parameters using the Cobas 6000 Analyzer with reagents and controls from the manufacturer (Roche Diagnostics, USA).

The evaluation of IL-6 and TNF cytokines was performed using the Cytometric bead array technique. The results were generated in graphs and tables with the aid of a specific software.

The 24-hour urine was used to dose urinary excretion of magnesium and creatinine. The patients were carefully instructed to: pack the urine in cleaned bottles of mineral water, discard the first urine of the initial day of collection and, from there, collect all urine produced during the 24-hour period and keeping it under refrigeration. Twenty-four-hour urine samples with a volume of less than 400mL or urinary creatinine <15mL/kg/24h (men) and <10mL/kg/24h (women) were considered due to the possibility of error in the collection.

To define CKD, two previous evaluations of renal function were considered, with a minimum interval of 3 months, according to the orientation of the KDIGO (Kidney Disease Improving Global Outcomes) (2012) [20]. GFR (glomerular filtration rate) was estimated using the formula derived from the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration**)** study [21] using creatinine as a reference for the calculation. From the results found, it was possible to obtain the staging of CKD.The dietary intake of magnesium was estimated by means of the 24-hour food recall (R24h) of three different days. In the measurement the participant reported all the food and beverages consumed, schedule, quantities in units of homemade measures, preparation form, as well as place of consumption (inside or outside the home). This record, based on the report of individuals to obtain data on the diet, provides detailed information about current food intake and, using repeated measures, can provide estimates of habitual intake [22]. After data collection, the R24h were reviewed to verify if the size of the food supply was in accordance with what is generally consumed by the Brazilian population. To quantify magnesium intake, the

 doi:10.20944/preprints202207.0198.v1

data obtained by R24h were collected in a spreadsheet of the Microsoft Office Excel software and the variables of interest were calculated based on the *Tabela Brasileira de Composição de Alimentos* [23]. The intake of macro and micronutrients was adjusted for energy using the residual method [24].

Anthropometric nutritional assessment was performed by measuring body mass, height, sagittal abdominal diameter (SAD) and waist circumference (WC), calf circumference (CC) and neck circumference (NC). Body mass was measured with the aid of a calibrated scale (Filizola®, Brazil) with a maximum capacity of 150kg and subdivisions per 100g and for height a portable statometer (Alturexata®, Brazil) with a scale of 0 to 220 cm and accuracy of 0.1 cm was used.

The weight-height assessment was performed using BMI and the classification proposed by the World Health Organization [25] for adults and Lipschitz (1994) [26] was adopted for the elderly.

The WC was measured at the midpoint between the last rib and the iliac crest, using an inelastic measuring tape (Sanny®, Brazil). NC was measured using an inelastic measuring tape (Sanny®, Brazil), measured at the base of the neck at the height of the cricothyroid cartila-gem [27]. CC was measured using an inelastic measuring tape (Sanny®, Brazil), in the maximum circumference in the plane perpendicular to the longitu-dinal line of the calf [28].

The SAD was measured in duplicate with a portable abdominal calibrator, with a subdivision of measurements of 0.1 cm (Holtain Kahn Abdominal Caliper®) [29].

The evaluation of the functional state of the skeletal muscle was performed by hand grip strength (HGS) with a manual hydraulic pressure dynamometer type Smedley (Jamar®), with a scale from 0 to 100kg and resolution of 1.0 kg, was used for the disease in the dominant arm [30].

Body composition was evaluated by Double X-Ray Emission Absorptiometry (DEXA) (Lunar DPX NT-GE healthcare®, Brazil) and Air Displacement Plethysmography (ADP) (BOD POD – COSMED®, Itália). The equipment used in the performance of the tests was calibrated daily, before the beginning of the measurements, following the recommendations of the manufacturers [31, 32].

DEXA is based on the attenuation by the patient's body of a radiation beam generated by an X-ray source with two energy levels. This beam crosses the individual in the remandoanterior direction and is captured by a detector. The software calculates the density of each sample from the radiation that reaches the detector at each energy peak [33]. The positioning in the equipment was done so that the sagittal line demarcated in this area passed under the center of the skull, spine, pelvis and legs [32]. The equipment used was lunar prodigi model – GE Healthcare® brand).

The PDA consists of a densitometric means of determining body composition, with the body weight obtained through the scale and the body volume provided by the application of gas laws inside two chambers. The technique estimates body volume based on Boyle's air displacement law, in which the volume varies inversely with the pressure while the temperature remains constant [33]. The estimation was performed using the BOD POD (BOD POD – COSMED®, Italy) device.

Initially the individual was weighed on a scale belonging to the BOD POD and then requested to enter into the measuring chamber and sit, remain immobile during the examination, and to perform three respiratory incursions, so that the equipment could measure the volume occupied by the patient, observing the Boyle principle [34]. Thus, the variations between pressure and volume were measured to determine body density. From this, body composition is measured, specifically the percentage of body fat (%BF) based on the Siri equation (1993) [35] through the specific software of the equipment itself.

To classify %BF, obtained through DEXA and PDA, we used the references established by Jackson and Pollock (1980) [36] according to gender and age group.

 doi:10.20944/preprints202207.0198.v1

2.2. Statistical analysis

Descriptive analyses were performed to characterize the patients. Categorical variables were presented by means of frequencies and percentages and quantitative variables by means of mean and standard deviation (mean ± SD) or median and interquartile difference (Quartile 3- Quartile1). The normality of the variables was tested by the Shapiro-Wilk test. The association between serum magnesium and 24-hour urine levels with CKD stages and study moments was performed by the ANOVA variance test.

A linear regression model with random effects was adjusted to investigate the impact of serum and urinary magnesium levels on body composition and in-flame state. For the effect of laboratory factors and food intake not confun-dissemthe analysis of the effect of serum and urinary magnesium, such factors were considered in the adjustment. The independent variables with a value of p <0.20 in the unadjusted analysis were included in the final model. The significance level considered in all analyses was 5%. The analyses were performed in the version 14 of the STATA (Data Analysis and Statistical Software) program.

3. Results

The sample consisted of 189 patients with CKD undergoing non-dialysis treatment followed at the CPDR-HUUFMA. The follow-up time was 24 months. During the follow-up period, there were 6 deaths (3.2%), mainly related to cardiovascular diseases. There were 49 censure due to progression of CKD to stage V with initiation of dialysis treatment (10.6%), diagnosis of cancer (3.2%), acquired immunodeficiency syndrome (0.5%) and refusal to participate in stages of the research (11.6%). One hundred, thirty-four patients (70.9%) remained until the end of the study. The mean age was 60.2±12.0 years and femaleindividuals (52.2%), married or in marital union (65.7%), black or brown (79.1%), with a maximum of eight years of schooling (92.5%) and family income of up to two minimum wages (48.5%) prevailed. Regarding lifestyle characteristics, 14.9% were etilists, 6.7% were smokers and 55.2% practiced physical activity. Hypertension was present in 88.8% of patients, 43.3% were diabetic and 72.4% were in stages 3A and 3B of CKD (Table 1).

The median Mg intake was 173 (120-238) mg/day for men and 204.5 (116-286) mg/day for women, with no statistically significant difference between genders (p=0.54). (Data not shown). There was no statistically significant difference between serum and urinary mg levels at different steps of data collection (p>0.05). The means of basal seric Mg, at 12months and at 24 months were 2.02±0.26mg/dL, 1.96±0.25mg/dL and 2.13±0.27mg/dL, respectively. Regarding urinary Mg, the mean values were: at baseline (71.82±35.53mg/dL), at 12 months (67.45±33.45mg/dL) and at 24 months (84.82±34.34mg/dL).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 July 2022 doi:10.20944/preprints202207.0198.v1

Table 1. Sociodemographic, lifestyle and clinical characteristics of patients with chronic kidney disease under non-dialysis treatment.

Variables	n	%
Age (years)		
20-44	14	4.2
45-59	36	11.0
≥ 60	84	84.8
Mean ±SD	60.2±12.0	
Sex		
Female	70	52.2
Marital Status		
Married/marital union	88	65.7
Single/separated/widowed	46	34.3
Self-referred color		
White	25	18.7
Black/brown	106	79.1
Others	3	2.2
Education		
≤ 8	124	92.5
> 8	10	7.5
Income (in minimum wages)		
< 1	36	26.9
≥1 and ≤ 2	65	48.5
>2	33	24.6
Alcohol consumption		
Present	20	14.9
Smoking		
Present	9	6.7
Physical activity		
Present	74	55.2
Arterial hypertension		
Present	119	88.8
Diabetes		
Present	58	43.3
Stages of chronic kidney disease		
3A	45	33.6
3B	52	38.8
4	37	27.6

There was no statistically significant difference between serum and urinary magnesium levels according to CKD progression (Table 2).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 July 2022 doi:10.20944/preprints202207.0198.v1

Table 2. Baseline, 12-month and 24-month baseline, 24-hour serum and urine magnesium levels of study participants by stage of chronic kidney disease

Variables	Serum magnesium Mean ± SD			24-hour urine magnesium Mean ± SD		
	Basal	**12 months**	**24 months**	**Basal**	**12 months**	**24 months**
Stage 3A	1.95±0.22	1.88±0.22	2.05±0.29	78.55±33.32	74.77±34.34	79.75±33.14
Stage 3B	2.02±0.28	1.99±0.25	2.12±0.23	71.01±37.50	67.33±34.88	94.83±37.26
Stage 4	2.04±0.25	2.00±0.26	2.21±0.29	66.10±33.80	61.54±33.45	77.66±29.38
p value*	*0.222*	*0.551*	*0.158*	*0.630*	*0.632*	*0.283*

*: Analysis of variance (ANOVA)

The coefficients of the longitudinal linear regression model of laboratory parameters and body composition associated with serum magnesium are shown in table 3.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 July 2022 doi:10.20944/preprints202207.0198.v1

Table 3. Longitudinal linear regression model of laboratory parameters and body composition associated with serum magnesium levels.

Variables	Unadjusted				Adjusted			
	β	CI [95%]		*p value*	β	CI [95%]		*p value*
TFGe[1], mg/min/1,73m²	-0.0020	-0.0031	-0.0031	**0.000**	-0.0006	-0.0007	-0.0020	0.345
RAC[2]	-0.0000	-0.0000	-3.4106	**0.028**	-0.0000	-0.0000	-5.1907	0.054
Calcium, mg/dL	0.0058	0.0001	0.0118	0.056	0.0044	0.0011	0.0101	0119
PTH[3], pg/mL	0.0008	0.0004	0.0012	**0.000**	0.0001	0.0003	0.0005	0.566
Alkaline phosphatase, mg/dL	0.0013	0.0004	0.0022	**0.003**	0.0006	0.0003	0.0016	0.166
HDL[4]-cholesterol, mg/dL	0.0020	0.0002	0.0037	**0.025**	0.0019	0.0002	0.0039	0.072
LDL[5]- cholesterol, mg/dL	-0.0009	-0.0015	-0.0002	**0.010**	-0.0004	-0.0011	-0.0002	0.224
Triglycerides, mg/dL	-0.0004	-0.0007	-0.0002	**0.000**	-0.0003	-0.0006	-0.0000	**0.022**
Uric acid, mg/dL	-0.0135	-0.0027	-0.0297	0.102	-0.0212	-0.0018	-0.0405	**0.032**
Interleukin 6, pg/mL	0.0051	0.0003	0.0105	0.066	0.0040	0.0011	0.0090	0.123
Mg food[6], mg/day	0.0025	0.0011	0.0038	**0.000**	0.0021	0.0078	0.0035	**0.002**
WC[7], cm	-0.0020	-0.0001	-0.0042	0.068	-0.0049	-0.0025	-0.0073	**0.000**
Lean mass, %	0.0022	0.0007	0.0052	0.137	0.0033	0.0000	0.0066	**0.046**
Body fat, %	-0.0025	-0.0056	-0.0005	0.103	-0.0038	-0.0073	-0.0004	**0.030**

[1]TFGe- estimated glomerular filtration rate; [2]RAC- albuminuria-creatinuria ratio; [3]PTH- parathyroid hormone; [4]HDL- high density lipoprotein; [5]LDL- low density lipoprotein; [6]Mg food- food consumption of magnesium; [7]WC – waist circumference.

In the unadjusted regression model, we found that serum magnesium was positively associated with phosphorus, alkaline phosphatase, HDL-c, parathyroid hormone and dietary Mg. Serum magnesium expressed a negative correlation with GET, albuminuria-creatininuria ratio, LDL-c and triglycerides.

In the adjusted model, phosphorus, uric acid, waist circumference, percentage of lean mass and dietary intake of magnesium were positively associated with serum magnesium. Triglyceride levels and fat mass percentage were negatively associated with serum magnesium.

Table 4 shows the coefficients of the longitudinal linear regression model of laboratory and body composition parameters associated with 24-hour urine magnesium. The unadjusted regression model showed that TFGe, hemoglobin, hematocrit, vitamin D, interleukin 6, magnesium dietary intake, WC, NC, CC, SAD, HGS and lean mass percentage expressed a positive correlation with 24-hour urine magnesium. The percentage of fat

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 13 July 2022 doi:10.20944/preprints202207.0198.v1

mass showed a negative correlation with magnesium in the urine of 24 hours. In the adjusted model, TFGe, IL-6, dietary magnesium intake and lean mass percentage showed a positive correlation with magnesium in 24-hour urine.

Table 4. Longitudinal linear regression model of laboratory parameters and body composition associated with 24-hour urine magnesium levels.

Variables	Unadjusted				Adjusted			
	β	CI [95%]		*p value*	β	CI [95%]		*p value*
TFGe[1], mg/min/1,73m²	0.4157	0.2763	0.5550	**0.000**	0.3096	0.1656	0.4536	**0.000**
ACR[2]	-0.0031	-0.0069	-0.0005	0.099	0.0002	0.0033	0.0038	0.894
Hemoglobin, g/dL	6.3571	4.4883	8.2260	**0.000**	5.2495	1.5899	12.0889	0.132
Hematocrit, %	1.9010	1.2757	2.5264	**0.000**	-0.6901	-2.9322	-1.5520	0.546
HDL[3]-cholesterol, g/dL	-0.1726	-0.3989	-0.0537	0.135	0.1846	0.0581	0.4274	0.136
Vitamin D, ng/mL	0.4786	0.2303	0.7269	**0.000**	0.1124	0.1503	0.3752	0.402
Interleukin 6, pg/mL	0.8390	0.1328	1.5452	**0.020**	0.7450	0.0292	1.4608	**0.041**
Mg alimentar[4], mg/day	0.0366	0.0010	0.0721	**0.043**	0.0362	0.0005	0.0719	**0.047**
BMI[5], kg/m²	0.5415	0.1696	1.2527	0.136	0.0010	1.8245	1.8266	0.999
WC[6], cm	0.3535	0.0705	0.6364	**0.014**	0.3763	0.2929	1.0456	0.270
Neck circumference, cm	1.6805	0.7975	2.5635	**0.000**	0.9440	2.3442	0.4561	0.186
Calf circumference, cm	1.1582	0.3090	2.0074	**0.008**	0.6825	0.5853	1.9505	0.291
SAD[7], cm	1.2383	0.2021	2.2746	**0.019**	1.7413	0.6455	4.1282	0.153
Hand grip strength , kg	1.0160	0.6181	1.4140	**0.000**	0.0212	0.6114	0.5688	0.944
Lean mass, %	0.7975	0.4210	1.1739	**0.000**	0.6910	0.1369	1.2450	**0.015**
Fat mass, %	-0.6756	-1.0207	-0.3304	**0.000**	-0.5708	-1.2931	-0.1514	0.121

[1] eGFR- estimated glomerular filtration rate; [2]ACR- albuminuria-creatinuria ratio; [3]HDL- high density lipoprotein; [4]Mg food-food consumption of magnesium; [5]BMI – body mass index; [6]WC – waist circumference; [7]SAD - Sagittal abdominal diameter.

4. Discussion

The aim of this study was to evaluate the association between serum and urinary levels of Mg and body composition and inflammatory markers in patients with non-dialysis CKD. To our knowledge, this was the first longitudinal study to bring together this set of methods with this population group.

 doi:10.20944/preprints202207.0198.v1

As it was hypothesized, the uric acid, percentage of lean mass and dietary intake of magnesium were positively associated with serum Mg. On the other hand, triglyceride, WC and fat mass percentage levels were negatively associated with serum Mg. In addition, TFGe, IL6, Mg food intake and lean mass percentage showed a positive association with urinary Mg levels.

The findings of this study demonstrate the clinical relevance of serum and urinary levels of Mg in the management of CKD patients undergoing dialysis. Mg deficiency is an emerging public health problem [37] and can be a risk factor for the development of kidney disease, cardiovascular complications [8], oxidative stress, pro-inflammatory state [7], obesity, metabolic syndrome, insulin resistance and hyperglycemia [38].

The reduction of magnesium levels is not uncommon among individuals with CKD, despite its low GFR. In a cross-sectional study of 5,181 patients with CKD in stages 1 to 5, hypomagnesemia, defined as serum Mg levels below 1.8 mg/dL, was one of the most prevalent electrolyte abnormalities [39]. On the other hand, studies with this population group have demonstrated adequate serum levels of Mg [40,41] corroborating the results of the current study that also showed adequate levels of Mg at baseline, after 12 months and after 24 months of follow-up.

The justification for this finding may be multiprofessional follow-up with nutritional guidelines aiming at the consumption of magnesium-sourced foods and mineral supplementation, when necessary. The correction of unhealthy diets is a priority to meet the recommended daily need of Mg. However, due to agronomic and environmental factors, as well as food processing, the Mg content in fruits and vegetables has dropped in the last 50 years and it may be necessary to complement it [42]. In clinical practice, serum magnesium dosage because it is more viable and low cost [43] is the most used laboratory test to assess mineral status. However, serum Mg does not correlate with the body Mg quantity because it is only 1% of the total Mg in the body [44]. This is one of the reasons why Mg deficiency is the most underestimated electrolyte imbalance in Western countries, where there is a significantly high risk of latent hypomagnesemia [45,46].

It has been proposed that urinary excretion of Mg should also be considered because the clinical deficiency of Mg is accurately diagnosed with a serum Mg value of less than 1.9 mg/dL with adequate urinary excretion of the mineral between 40-80 mg/day [47,48]. In the present study, urinary mg levels were measured and showed mean values within the normal range at baseline and during the follow-up period, with no statistically significant difference ($p>0.05$) in the different periods. However, the discussion about the reference values for Mg is still ongoing, since there are discrepancies between the clinical symptoms of Mg deficiency and the serum and urinary concentration threshold of the mineral [49].

Moderate or subclinical deficiency of Mg induces a low-grade chronic inflammation sustained by the release of inflammatory cytokines and production of free radicals, which exacerbate a pre-existing inflammatory state [7]. For this reason, it is considered a risk factor for pathological conditions characterized by chronic inflammation, such as CKD, hypertension, cardiovascular disorders, obesity, metabolic syndrome and diabetes [50,51]. In this study, IL6 levels showed a positive association with urinary excretion of Mg, corroborating the findings of Simental-Mendial et al (2017) [52] which in studies with humans and animal models showed Mg deficiency as an beginning for the inflammatory process. Veronese et al (2022), in a systematic review with meta-analysis, [53] demonstrated the reduction of inflammatory markers when Mg levels were adequate.

One possible explanation is that hypomagnesemia stimulates macrophages and the influx of calcium ions into cells. Increased levels of cellular calcium increase the Mg needed to block the influx of calcium ions with increased stimulation of N-methyl-D-aspartate receptors with high calcium permeability [50]. Thus, the stimulation of these receptors leads to the opening of non-selective channels for cations with increased calcium ions in neuronal cells and release of neurotransmitters and cytokines such as IL6 [54].

Mg status may affect body composition, evidence suggests that there is a negative association between body fat percentage and serum Mg levels in obese individuals [38]. In the

 doi:10.20944/preprints202207.0198.v1

present study, serum Mg levels were negatively associated with body fat and WC percentage. Corroborating our results, the NHANES study highlights that mg deficit is more prevalent in Americans with BMI in the obesity range [14]. Similarly, the 30-year-old CARDIA longitudinal study, conducted in more than 5,000 individuals, concluded that Mg intake is inversely associated with obesity incidence and CRP levels [15]. In addition, other studies have concluded an inverse association between Mg intake and adiposity markers, such as BMI and WC [55,56].

Mg plays an important role in controlling cell proliferation [57], protein synthesis [58,59] and effects on the secretion or action of anabolic hormones [60]. These lean mass-related functions were identified by regression analysis of the current study, which revealed a positive correlation between lean mass and serum and urinary mg levels. In this sense, a randomized placebo-controlled study revealed that supplementation of overweight women with 250 mg of Mg daily for eight weeks resulted in an increase in lean body mass and a decrease in fat mass compared to baseline values [61]. Another cross-sectional study with 396,283 participants concluded that a higher mg intake was associated with a lower chance of decreased strength and muscle mass [62]. It is noteworthy that no studies were evidenced with CKD patients.

Another interesting result evidenced in our study was a negative correlation between triglyceride levels and serum Mg. A consistent association between magnesium concentrations and lipid profile has not yet been demonstrated in the literature. It is known that in the liver a decrease in mg activity and pyruvate dehydrogenase enzyme can divert glucose metabolism to the oxidative phase of the phosphate pentoses pathway, thus generating an excess of NADPH [63] that provides redox potential for fatty acid biosynthesis, promoting an increase in the synthesis of triglycerides and LDL cholesterol [64]. A meta-analysis published by Simental-Mendia et al (2017) [52] showed in subgroups with hypercholesterolemia and hypertriglyceridemia the reducing effect of Mg supplementation on LDL cholesterol and triglyceride levels. Subsequently, a randomized study published by Rodriguez-Moran et al. (2018) [65] demonstrated a beneficial effect of oral magnesium supplementation on triglyceride concentrations in individuals with metabolic syndrome.

In the current study, a positive correlation was observed between the food intake of Mg and its serum and urinary concentrations. In fact, the serum and urinary concentrations of Mg depend, among others, on the daily intake of this mineral [46]. In the results of the NHANES, 45% of the American population presented food deficiency in mg intake [66], and other population studies also point to inadequate intake of this mineral [55, 67].

The American National Academy of Medicine recommends the consumption of 310-360 mg and 400-420 mg for women and adult men, respectively [68]. While drinking water accounts for about 10% of the daily intake of Mg [69] chlorophyll is the main source of this mineral. Nuts, seeds and unprocessed cereals are also rich in Mg [70]. It is noteworthy that processed foods have a lower Mg content when compared to whole grains and that the dietary intake of this mineral in the Western world is decreasing due to the consumption of processed foods [71].

On the other hand, the progressive decline of TFG in patients with CKD is associated with a significant reduction in appetite and food intake, which may impair mg intake [72,73]. In the present study, the median mg consumption was below that recommended for men and women, with no statistically significant difference between genders. Of the total dietary Mg consumed, approximately 11 to 65% is absorbed into the gastrointestinal tract and the remainder is eliminated in feces [74]. As CKD progresses, the absorption of nutrients into the gastrointestinal tract eventually becomes abnormal due to uremia that affects the microbiome and destroys the intestinal epithelium. In addition, active vitamin D, which can increase intestinal absorption of Mg, is decreased in CKD patients [75].

In the present study, a negative correlation was also observed between uric acid levels and serum Mg. Uric acid is the final product of purine metabolism, with an increase in serum urate concentration as TFG decreases, triggering several physiological mechanisms, such as inflammation, oxidative stress and endothelial injury [76]. There is an essential and potentially modifiable relationship between Mg intake and serum uric acid

levels [77,78]. The NHANES data with 37,215 individuals showed a negative correlation between Mg consumption and hyperuricemia, suggesting that deficient intake of this mineral may increase the risk of hyperuricemia [79].

Finally, another result of the study was a positive correlation between TFG and urinary excretion of Mg. In fact, the kidneys are crucial in Mg homeotase, because their serum concentration is mainly controlled by excretion in urine. In moderate CKD, the increase in mg excretion fraction compensates for the loss of glomerular filtration, so that serum Mg levels are kept within the normal range [80]. Based on the information presented, more studies are needed to validate and unify these findings that may be useful in the development of better, more appropriate and personalized management of CKD patients in the non-dialysis phase.

This study has some limitations: 1) It is carried out in a single center with a small sample, which, however, is statistically representative of the population studied; 2) As some patients had advanced kidney disease it was not possible to discontinue diuretics for more than 24 hours at the risk of clinical decompensation.

Thestrength of the current research was the fact of evaluating serum, urinary levels and food intake of Mg in patients diagnosed with CKD in the non-dialysis phase. In addition, methods considered gold standard were used in the evaluation of body composition. The longitudinal approach also contributed to the robustness of the study.

5. Conclusions

The results of this longitudinal study demonstrated a positive association of serum and urinary mg levels with lean body mass and negative correlation of serum Mg with indicators of total and central body fat. In addition, the marker of interleukin inflammation 6 showed a positive relationship with urinary magnesium levels.

Author Contributions: Author Contributions: Conceptualization, D.R.S.C and B.D.J.A.; methodology, D.R.S.C.; software, E.J.F.S; validation, D.R.S.C., S.F.N. and B.F.M.; formal analysis, M.C.A; investigation, D.R.S.C.; resources, B.D.J.A., S.E.M. and S.F.N.; data curation, D.R.S.C.; writing—original draft preparation, D.R.S.C.; writing—review and editing, B.D.J.A., S.E.M., C.E.C.R.L., E.J.F.S. and M.C.A.; visualization, A.D.V.; supervision, A.D.V., S.F.N. and B.F.M.; project administration, S.F.N. and B.F.M.; funding acquisition, S.F.N. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of Hospital Universitário da Universidade Federal do Maranhão (parecer: 2.727.940)

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Akchurin, O.M. Chronic Kidney Disease and Dietary Measures to Improve Outcomes. *Pediatric Clin North Am* ***2019***, *66*, pp. 247-267. DOI: https://doi.org/10.1016/j.pcl.2018.09.007
2. Stevens, P.E.; Levin, A. Evaluation and management of chronic kidney disease: synopsis of the kidney disease: improving global outcomes 2012 clinical practice guideline. *Annals of Internal Medicine* **2013,** 158, pp. 825-830. DOI: https://doi.org/10.7326/0003-4819-158-11-201306040-00007
3. Czaja-Stolc, S.; Potrykus, M., Stankiewicz, M., Kaska, L.; Małgorzewicz, S. Pro-Inflammatory Profile of Adipokines in Obesity Contributes to Pathogenesis, Nutritional Disorders, and Cardiovascular Risk in Chronic Kidney Disease. *Nutrients* **2022,** 14, pp. 1457. DOI: https://doi.org/10.3390/nu14071457
4. Martin-Taboada, M.; Vila-Bedmar, R.; Medina-Gómez, G. From Obesity to Chronic Kidney Disease: How Can Adipose Tissue Affect Renal Function? *Nephron* **2021**, 145, pp. 609–613. DOI: 10.1159/000515418

5. Silva, M. I. B.; Klein, M. R.; Cardoso, E. S.; Costa, M. S.; Martins, C. J.; Bregman, R. Synergistic effect of inflammatory cytokines and body adiposity on insulin resistance and endothelial markers in patients with stages 3-5 chronic kidney disease. *Journal of Renal Nutrition* **2020**, 30, pp. 36-45. DOI: 10.1053/j.jrn.2019.02.001
6. Krane, V.; Wanner, C. Statins, inflammation and kidney disease. *Nature Reviews Nephrology* **2011**, 7, pp. 385-97. DOI: 10.1038/nrneph.2011.62
7. Nielsen, F. H. Magnesium deficiency and increased inflammation: current perspectives. *J Inflamm Res* **2018**, 11, pp. 25-34. DOI: 10.2147/JIR.S136742
8. Tin, A. et al. Results from the Atherosclerosis Risk in Communities study suggest that low serum magnesium is associated with incident kidney disease. *Kidney International* **2015**, 87, pp. 829-837. DOI: 10.1038/ki.2014.331
9. Dominguez, L.J; Veronese, N.; Guerrero-Romero, F.; Barbagallo, M. Magnésio em Doenças Infecciosas em Pessoas Idosas. *Nutrientes* **2021**, 13 :180. DOI: 10.3390/nu13010180
10. Liu, M.; Dudley, S.C. Magnesium, oxidative stress, inflammation, and cardiovascular disease. *Antioxidants* **2020**, 9, pp. 907. DOI: 10.3390/antiox9100907
11. Piuri, G.; Zocchi, M.; Della Porta, M.; Ficara, V.; Manoni, M.; Zuccotti, G.V.; Pinotti, L; Maier, J; Cazzola, R. Magnesium in obesity, metabolic syndrome, and Type 2 diabetes. *Nutrients* **2021**, 13, pp. 320. DOI: 10.3390/nu13020320
12. Oliveira, A.R. et al. Magnesium status and its relation ship with c-reactive protein in obese women. *Biological Trace Element Research* **2015**, 168, pp. 296-302. DOI: 10.1007/s12011-015-0358-8
13. Cruz, K.J. et al. Influence of magnesium on insulin resistance in obese women. *Biological Trace Element Research* **2014**, 160, pp. 305-310. DOI: 10.1007/s12011-014-0044-2
14. Jiang, S. et al. Association between dietary mineral nutrient intake, body mass index, and waist circumference in US adults using quantile regression analysis NHANES 2007–2014. *PeerJ* **2020**, 8, pp. 9127. DOI: https://doi.org/10.7717/peerj.9127
15. Lu, L. et al. Magnesium intake is inversely associated with risk of obesity in a 30-year prospective follow-up study among American young adults. *Eur J Nutr* **2020**, 59, pp. 3745–3753. DOI: https://doi.org/10.1007/s00394-020-02206-3
16. Travassos, C.; Williams, D. R. The concept and meansurement of race and their relationship to public health: a review focused on Brazil and the United States. *Cadernos de Saúde Pública* **2004**, 20, pp. 660-678. DOI: 10.1590/s0102-311x2004000300003
17. Matsudo, S. et al. Questionário Internacional de Atividade Física (IPAQ): estudo de validade e reprodutibilidade no Brasil. Revista Brasileira de Atividade Física e Saúde **2001**, 6, pp. 5-18. DOI: https://doi.org/10.12820/rbafs.v.6n2p5-18
18. Barroso WKS, Rodrigues CIS, Bortolotto LA, Mota-Gomes MA, Brandão AA, Feitosa ADM, et al. Diretrizes Brasileiras de Hipertensão Arterial – 2020. Arquivos Brasileiros de Cardiologia 2021; 116(3): 516-658. DOI:10.36660/abc.20201238
19. Friedewald, W.T.; Levi, R.I.; Fredrickson, D.S. Estimation of the concentration of low density lipoproteins cholesterol in plasma without use of the ultracentrifuge. *Clinical Chemistry* **1972**, 18, pp. 499-502.
20. KDIGO 2012. Kidney Disease Improving Global Outcomes. Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. *Kidney International Supplements* **2013**, 3, pp. 5-14.
21. Levey, A. R. et al. For the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A New Equation to Estimate Glomerular Filtration Rate. *Annals of Internal Medicine* **2009**, 150, pp. 604-612.
22. Tucker, K. L. Evaluation of dietary intake in population-based studies of gene-diet interaction. *Nutrition, Metabolism and Cardiovascular Diseases* **2007**, 17, pp. 74-81. DOI: 10.1016/j.numecd.2006.07.010.
23. Unicamp. Tabela brasileira de composição de alimentos–TACO. *Nepa* **2011**, pp. 57.
24. Willett, W.; Stampfer, M.J. Total energy intake: implications for epidemiologic analyses. *Am J Epidemiol.* **1986**, 124, pp. 17-27. DOI: 10.1093/oxfordjournals.aje.a114366
25. World health organization (WHO). Physical Status: the use and interpretation of anthropometry. Report of a WHO Expert Committee, n. 854. *Geneva* **2000**.
26. Lipschitz, D. A. Screening for nutritional status in the elderly. *Primary Care* **1994**, 21, pp. 55-67.
27. Ben-Noun, L. L.; Laor, A. Relationship between changes in neck circumference and cardiovascular risk factors. *Experimental & Clinical Cardiology* **2006**, 11, pp. 14-20.
28. Lohman, T.G; Roche, A.F; Martorel, R. Anthropometrics standartization reference manual. *Champaign:* Human Kinetics Books **1988**.
29. Carneiro Roriz, A. K. et al. Methods of predicting visceral fat in Brazilian adults and older adults: a comparison between anthropometry and computerized tomography. *Archivos Latinoamericanos de Nutricion* **2011**, 61, pp. 5-12.
30. Schlüssel, M. M. et al. Reference values of handgrip dynamometry of healthy adults: a population-based study. *Clinical Nutrition* **2008**, 27, pp. 601-607. DOI: 10.1016/j.clnu.2008.04.004
31. Lewiecki, E.M. et al. Melhores práticas para medição e relatórios de absorciometria de raios X de dupla energia: International Society for Clinical Densitometry Guidance. *Journal of Clinical Densitometry* **2016**, 19, pp. 127-140.
32. Mccrory, M.A. et al. Evaluation of a new air displacement plesthysmograph for measuring human body composition. *Medicine and Science in Sports and Exercise* **1995**, 27, pp. 1686-1691.
33. Laskey, M.A. et al. Comparison of the effect of different reference data on Lunar DPX and Hologic QDR-1000 dual-energy X-ray absorptiometers. *British Journal of Radiology* **1992**, 65, pp. 1124-1129.

34. Garrow, J.S. et al. A new method for measuring the body density of obese adults. *British Journal of Nutrition* **1979**, 42, pp. 173-83.
35. Siri, W. E. Body composition from fluid spaces and density: analysis of methods. 1961. *Nutrition* **1993**, 9, pp. 480.
36. Jackson, A. S.; Pollock, M. L. Generalized equations for predicting body density of women. *Medicine and Science in Sports and Exercise* **1980**, 12, pp. 175-183.
37. Rosanoff, A.; Weaver, C.M; Rude, R.K. Suboptimal magnesium status in the United States: are the health consequences underestimated? *Nutr Rev.* **2012**, 70 pp. 153-641. DOI: 10.1111/j.1753-4887.2011.00465.x.
38. Morais, J.B.S.; Severo, J.S.; Dos Santos, L.R.; De Sousa Melo, S.R.; De Oliveira Santos, R.; De Oliveira, A.R.S.; Cruz, K.J.C.; Do Nascimento Marreiro, D. Role of Magnesium in Oxidative Stress in Individuals with Obesity. *Biol. Trace Elem. Res.* **2017**, 176, pp. 20–26. DOI: 10.1007/s12011-016-0793-1
39. Oka, T. et al. A perda renal de magnésio associada à proteinúria leva à hipomagnesemia: uma anormalidade eletrolítica comum na doença renal crônica. *Transplante Nephrol Dial.* **2019,** 34, pp. 1154–1162. DOI: 10.1093/ndt/gfy119
40. Dias, R. S. C. et al. Correlation Between Parathyroid Hormone Levels with Urinary Magnesium Excretion in Patients with Non-Dialysis Dependent Chronic Kidney Disease. *International journal of nephrology and renovascular disease* **2020**, 13, pp. 341. DOI: 10.2147/IJNRD.S282106
41. Ferrè, S. et al. Low serum magnesium is associated with faster decline in kidney function: the Dallas Heart Study experience. *Journal of Investigative Medicine* **2019**, 67, pp. 987-994. DOI: 10.1136/jim-2018-000966
42. Cazzola, R.; Della Porta, M.; Manoni, M.; Iotti, S.; Pinotti, L.; Maier, J.A. Going to the roots of reduced magnesium dietary intake: A tradeoff between climate changes and sources. *Helyon* **2020**, 6, pp. 05390.
43. Oh, H.E.; Deeth, H.C. Magnesium in milk. *Int. Dairy J.* **2017**, 71, pp. 89–97. DOI: https://doi.org/10.1016/j.idairyj.2017.03.009
44. Razzaque, M.S. Magnesium: Are We Consuming Enough? *Nutrients* **2018**, 10, pp. 1863. DOI: https://doi.org/10.3390/nu10121863
45. Nielsen, F.H. The Problematic Use of Dietary Reference Intakes to Assess Magnesium Status and Clinical Importance. *Biol. Trace Elem. Res.* **2019**, 188, pp. 52–59. DOI: 10.1007/s12011-018-1573-x
46. Gröber, U.; Schmidt, J.; Kisters, K. Magnesium in prevention and therapy. *Nutrients* **2015**, 7, pp. 8199–8226. DOI: 10.3390/nu7095388
47. Malinowska, J.; Małecka, M.; Ciepiela, O. Variations in Magnesium Concentration Are Associated with Increased Mortality: Study in an Unselected Population of Hospitalized Patients. *Nutrients* **2020**, 12, pp. 1836. DOI: 10.3390/nu12061836.
48. Costello, R.B. et al. Perspective: The Case for an Evidence-Based Reference Interval for Serum Magnesium: The Time Has Come. *Adv. Nutr. Int. Rev. J.* **2016**, 7, pp. 977–993.
49. Liebscher, D.H.; Liebscher, D.E. About the misdiagnosis of magnesium deficiency. *J. Am. Coll. Nutr*. **2004,** 23, pp. 730S–731S. DOI: 10.1080/07315724.2004.10719416
50. Maier, J.A.; Castiglioni, S.; Locatelli, L.; Zocchi, M.; Mazur, A. Magnesium and inflammation: Advances and perspectives. *Semin. Cell Dev. Biol*. **2020**. DOI: https://doi.org/10.1016/j.semcdb.2020.11.002
51. Dinicolantonio, J.J.; O'keefe, J.H.; Wilson, W. Subclinical magnesium deficiency: A principal driver of cardiovascular disease and a public health crisis Coronary artery disease. *Open Hear*. **2018**, 5, pp. 668. DOI: https://doi.org/10.1136/openhrt-2017-000668
52. Simental-Mendia, L.E; Sahebkar, A.; Rodriguez-Moran, M.; Zambrano-Galvan, G.; Guerrero-Romero, F. Efeito da Suplementação de Magnésio nas Concentrações de Proteína C-Reativa no Plasma: Uma Revisão Sistemática e Meta-Análise de Controlado Randomizado Ensaios. atual Farmácia. *Des.* **2017**, 23, pp. 4678–4686. DOI: 10.2174/1381612823666170525153605.
53. Veronese, N. et al. Effect of Magnesium Supplementation on Inflammatory Parameters: A Meta-Analysis of Randomized Controlled Trials. *Nutrients* **2022**, 14, pp. 679. DOI: 10.3390/nu14030679
54. Asemi Z., Karamali M., Jamilian M., Foroozanfard F., Bahmani F., Heidarzadeh Z., Benisi-Kohansal S., Surkan PJ, Esmaillzadeh A. A suplementação de magnésio afeta o estado metabólico e os resultados da gravidez no diabetes gestacional: Um estudo randomizado, duplo-cego, controlado por placebo. *J. Clin. Nutr.* **2015**, 102, pp. 222-229.
55. McKay, J.; Ho, S.; Jane, M.; Pal, S. Overweight & obese Australian adults and micronutrient deficiency. *BMC Nutr.* **2020**, 6, pp. 12.
56. Castellanos-Gutiérrez, A.; Sánchez-Pimienta, T.G.; Carriquiry, A.; Da Costa, T.H.M.; Ariza, A.C. Higher dietary magnesium intake is associated with lower body mass index, waist circumference and serum glucose in Mexican adults. *Nutr. J.* **2018**, 17, pp.114. DOI: https://doi.org/10.1186/s12937-018-0422-2
57. Wolf, F.I.; Cittadini, A. Magnesium in cell proliferation and differentiation. *Front. Biosci.* **1999**, 4, pp. D607–D617. DOI: 10.2741/Wolf.
58. De Baaij, J.H.; Hoenderop, J.G.; Bindels, R.J. Magnesium in man: Implications for health and disease. *Physiol. Rev.* **2015**, 95, pp. 1–46. DOI: 10.1152/physrev.00012.2014.
59. Dorup, I.; Clausen, T. Effects of magnesium and zinc deficiencies on growth and protein synthesis in skeletal muscle and the heart. *Br. J. Nutr.* **1991**, 66, pp. 493–504. DOI: 10.1079/BJN19910050.
60. Maggio, M. et al. Magnesium and anabolic hormones in older men. *Int. J. Androl.* **2011**, 34, pp. 594–e600. DOI: 10.1111/j.1365-2605.2011.01193.x

61. Moslehi, N; Vafa, M; Sarrafzadeh, J; Rahimi-Foroushani, A. Does magnesium supplementation improve body composition and muscle strength in middle-aged overweight women? A double-blind, placebo-controlled, randomized clinical trial. *Biol Trace Elem Res.* **2013**,153, pp. 111-118. DOI: 10.1007/s12011-013-9672-1.
62. Petermann-Rocha, F. et al. Factors associated with sarcopenia: a cross-sectional analysis using UK Biobank. *Maturitas* **2020**, 133, pp. 60-67. DOI: 10.1016/j.maturitas.2020.01.004
63. Maguire, D.; Talwar, D.; Shiels, P.G.; McMillan, D. The role of thiamine dependent enzymes in obesity and obesity related chronic disease states: A systematic review. *Clin. Nutr. ESPEN* **2018**, 25, 8–17. DOI: 10.1016/j.clnesp.2018.02.007
64. Deepti, R.; Nalini, G. Anbazhagan. Relationship between hypomagnesemia and dyslipidemia in type 2 diabetes mellitus. *Asian J. Pharm. Res. Health Care* **2014**, 6, pp. 32–36.
65. Rodriguez-Moran, M.; Simental-Mendia, L.E.; Gamboa-Gomez, C.I.; Guerrero-Romero, F. Oral Magnesium Supplementation and Metabolic Syndrome: A Randomized Double-Blind Placebo-Controlled Clinical Trial. *Adv. Chronic Kidney Dis.* **2018**, 25, pp. 261–266. DOI: 10.1053/j.ackd.2018.02.011
66. Moshfegh Alanna, G.J.; Jaspreet, A.; Donna, R.; Randy, L. What We Eat in America, NHANES 2005–2006: Usual Nutrient Intakes from Food and Water Compared to 1997 Dietary Reference Intakes for Vitamin D. Calcium, Phosphorus, and Magnesium. *US Department of Agriculture, Agricultural Research Service; Washington*, DC, USA: 2009.
67. Choi, M.K.; Weaver, C.M. Daily Intake of Magnesium and its Relation to Urinary Excretion in Korean Healthy Adults Consuming Self-Selected Diets. *Biol. Trace Elem. Res*. **2017**, 176, pp. 105–113. DOI: 10.1007/s12011-016-0822-0.
68. Saris, N.E. et al. An update on physiological, clinical and analytical aspects. *Clin Chim Acta.* **2000,** 294, pp. 1–26. DOI: 10.1016/s0009-8981(99)00258-2.
69. Marx, A; Neutra, R.R. Magnesium in drinking water and ischemic heart disease. *Epidemiol Rev*. **1997**, 19, pp. 258–272.
70. Fox, C.; Ramsoomair, D.; Carter, C. Magnesium: its proven and potential clinical significance. *South Med J.* **2001**, 94, pp. 1195–1201.
71. Ford ES, Mokdad AH. Dietary magnesium intake in a national sample of US adults. *J Nutr.* **2003**, 133, pp. 2879–2882. DOI: 10.1093/jn/133.9.2879.
72. Yuan, Q. et al. Urinary magnesium predicts risk of cardiovascular disease in Chronic Kidney Disease stage 1–4 patients. *Clinical Nutrition* **2021**, 40, pp. 2394-2400. DOI: 10.1016/j.clnu.2020.10.036
73. Armstrong, J.E; Laing, D.G.; Wilkes, F.J.; Kainer, G. Smell and taste function in children with chronic kidney disease. *Pediatr Nephrol* **2010**, 25, pp. 1497e504. DOI: 10.1007/s00467-010-1529-7
74. Fine, K. D.; Santa Ana, C. A.; Porter, J. L.; Fordtran, J. S. Intestinal absorption of magnesium from food and supplements. *The Journal of clinical investigation* **1991**, 88, pp. 396-402.
75. Vaziri, N.D; Yuan, J.; Norris, K. Role of urea in intestinal barrier dysfunction and disruption of epithelial tight junction in chronic kidney disease. *Am J Nephrol* **2013**, 37, pp. 1-6. DOI: 10.1159/000345969
76. Johnson, R. J. et al. Hyperuricemia, acute and chronic kidney disease, hypertension, and cardiovascular disease: report of a scientific workshop organized by the National Kidney Foundation. *American Journal of Kidney Diseases* **2018**, 71, pp. 851-865. DOI: 10.1053/j.ajkd.2017.12.009
77. Wang, Y. et al. Association between dietary magnesium intake and hyperuricemia. *Plos One* **2015**, 10, pp. 0141079. DOI: 10.1371/journal.pone.0141079
78. Navin, S.; Krishnamurthy, N.; Ashakiran, S.; Dayanand, C.D. The association of hypomagnesaemia, high normal uricaemia and dyslipidaemia in the patients with diabetic retinopathy. *J. Clin. Diagn. Res.* **2013**, 7, pp. 1852–1854. DOI: 10.7860/JCDR/2013/6106.3332
79. Zhang, Y.; Qiu, H. Dietary magnesium intake and hyperuricemia among US adults. Nutrients **2018**, 10, pp. 296. DOI: 10.3390/nu10030296
80. Noiri, C. et al. Clinical significance of fractional magnesium excretion (FEMg) as a predictor of interstitial nephropathy and its correlation with conventional parameters. *Clin Exp Nephrol.* **2015**, 19, pp. 1071-1078. DOI: 10.1007/s10157-015-1099-x